Control Ability of Extracts from *Ajuga* Plants on Some Pests*

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ABSTRACT Several phytoecdysteroids have been found in the extracts of *A. multiflora* and *A.linearifolia* using HPLC analysis. Those were 20-OH ecdysone, cyasterone, 3-acetate-20-OH ecdysone, 2-acetate-20-OH ecdysone, ajugalactone. We found out that those phytoecdysteroids could lead the second instar larva of *Clostera anastomosis* and *Stilpnotia candida* to death. The modified mortality rate was from 44.64% to 96.24%. Those extracts could significantly reduce the amount of nymph produced by the female *Tuberolachnus salignus*. The number of the nymph produced after treating with those extracts was only 20% to 70% of that produced by the untreated group. The death rate of the newly produced nymph which treated was among 33.33% to 58.82%. It was much higher than the death rate of the control group (17.57%).
Key words: *Ajuga*, Phytoecdysteroids, Control, *Clostera anastomosis*, *Stilpnotia candida*, *Tuberolachnus salignus*

Introduction

During approximately 200 million years' coexistence, plant and animal kingdoms have affected each other in different ways. Many plants produce allelochemicals (secondary plant substances) which affect on the life of herbivores. Some of the allelochemicals can interfere with the hormonal balance of the insect development inducing insect developmental inhibitor type symptoms.

Since the isolation of the first moulting hormone from a plant, *Podocarpus nakai* (Podocarpaceae), a number of phytoecdysones has been found in plants. These phytoecdysones have significant physiological effects on insects and have there for been suggested to play a role in the defense of plants from insect attack[13].

Ajuga species produce significant quantities of diterpenoid neo-clerodans (e.g. ajugarins, ajugareptansin, ajugareptansons, ajugapitins etc.) with phago-deterrent activity and phytoecdysteroids (e.g. 20-OH ecdysone, cyasterone, sengosterone, ajugalactone, etc.) with insect developmental inhibitor type activities, see Table 1 (From Dr. Bela Darvas et al. 1993).

The purposes of this study were to find out those phytoecdysteroids contained in the *Ajuga multiflora* Bunge and *A. linearifolia* Pamp. , and to find out the effects of those extracts on some of the forest pests.

Materials and Methods

The two species of *Ajuga* analyzed for phytoecdysone contents were *Ajuga linearifolia* (collected from Nanjing City, Jiangshu Province) and *A. multiflora* (Collected from Anshan City, Liaoning Province).

Whole or the aerial part of *Ajuga* or the aerial part of plants in summer, at flowering were cleaned with water and dried at 70 °C. Dry plant materials was ground with Labor Min grinder. Ten gram ground materials were extracted in 180 mL methanol with ultraturax TP 45. Extraction was repeated twice in 180 mL methanol. Solvent was evaporated under vacuum at 60°C. A single sample was dissolved in 10 mL ethanol(1g dry ma-

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terial/mL conc.) and used for analyzing or in biological assays.

Table 1. Phytoecdysteroids in *Ajuga* species, mg. kg^{-1}

Ajuga species	Tissue	20-OH ecdysone	cyasterone	makisterone A	Ajugasterone				ajugalactone	Others	Ref. No.
					A	B	C	D			
A. australis		+	+	+							2
A. bracteosa	l	+	+								12
	l	+	+				+				13
A. chamaecystus	l	+	+				+				13
A. chamaepity	w,d	38	160	40					43		5
A. chia	l, s, f		19								9
	w	100									1
A. decumbens	w,f	1200	800			10	15				15
	w	1250	600			15	15		100		11
A. incisa	w,f	1200	800		140	80					15
A. iva	w,f	121					+				9
	w,f	50	1								10
	w,d			1070							10
	r,d	2200	1330	280						23-OH-CY	16
	s,d	800	530	640							16
	l,d	520	440	440							16
	w,d	1600	1300	1600						22-OXO-CY	19
										24(28)MKA	19
										24PRECY	19
A.Iva var. psudoiva		44	70	35							7
A. japonica	l,d	1800	580				130				15
A. nipponensis	w,f	1200	800								15
	w,d	1000			1000			1000		STD:1000	6
A. pyramidalis											
spp. occidentalis		170	70	50				90		NC:180	7
A. reptans	w,d	66	26						94	NC:120	3
										NS:110	3
	l	+									13
	w,d									2Ac-cy	4
										3Ac-cy	4
var. atropurpurea	r, f	47	15							NC:21	14
										ICY:5	14
turkestanica		480	100				+			TU:520	17
										22Ac-cy-500	18

Notes: TU=turkesterone, NC=29-norcyasterone, ICY=Isocyasterone, SG=sengosterone, NS=29-norsengosterone, 24(28)MKA=24(28)-dehydro-MKA, 24PRECY=24-dehydro-PRECY, STD=stachisterone D, 2Ac-CY=2-acetyl-CY, 3Ac-CY=3-caetyl-CY, 22Ac-CY=22-acetyl-Cy, l=leaves, w=Whole plants, r=roots, d= dried plants, s=stems, *A. bracteosa* Benth. (Wall. Pl. As. Rar., i. 59)= *A. remota, A. chamaecystus* Ging. (Bent. Lab. Gen. Sp., 698.)=*A. camaecistus* (K.H. Rechinger, Fl. Iran, 150:11)= *A. chameacistus* (Kubo et al., 1983).

Determination of phytoecdysone

One mL ethanolic sample was evaporated under N_2 at room temperature. Sep-pak C_{18} column was cleaned and activated with 10 mL double distilled, deionised water. The sample was dissolved in 5 mL distilled water twice and put into the column. The sample in the column was cleaned with 10 mL 15% methanol. Phytoecdysteroids was collected with 5 mL 85% methanol and the sample was dried under N_2.

Applied Biosyst. 491 Dynamic Mixer Injector, Applied Biosyst. 400 Solvent Delivery system (1.2 mL/min, isopropylalcohol: water=7:93), 10 ×0.4cm I. D. (5 *u*m) Spherisorb ODS-2 C_{18} (Tracer analitica) column, Spark Holland Column Thermostat (55 ℃), Applied Biosyst. 1000s Diode Detector (Standard: methylanthranilate) were used for determination.

Biological assays

Larvae of *Clostera anastomosis* (Linnaeus) and *Stilpnotia candida* Staudinger The larvae used were gotten from the *Populus* stands in Dalian, Liaoning Province. Those larvae were weighted and measured individually to make sure that all of those larvae used in these experiments were in the same instar.

Twenty second instar larvae were reared in each glass pot that was 10 cm in diameter and 15 cm high. The food used to feed those larvae were fresh leaves on their twig of *Populus simonii ×nigra*. Those leaves were soaked in the diluted extracts (diluted 25, 50, 100 times) originated from different species for 3 s, air dried, water cultivated in a small glass tube. Those pots were covered with fine gauze after those animals and their food have been put in them. Put those pots into a constant temperature animal rearing room at 28 °C and 60%-70% R.H., under light:dark conditions 16:8 h. Ten mL 100% ethanol diluted 25 times were used as control groups . Three repetition was used in each treatment. The foods for those larvae were changed every two to three days and at the same time the number of dead larvae was recorded.

Larvae of *Tuberolachnus salignus* (Gmelin):

The nymphs were collected from the *Salix fragilis* Linneaus stands in the Dalian, Liaoning Province. Ten female adults were inoculated on the small branches of *Salix fragilis* cultivating in diluted (diluted 25, 50, 100 times) extracts from *Ajuga*. plants. Put all of those animals and their food into a pot which is 10 cm in diameter and 15 cm high, then cover it immediately with fine gauze. Put those treated animals in to a animals rearing room. The rearing condition was the same as used in the rearing of larvae of *Clostera anastomosis* and *Stilpnotia candida* . Ten mL 100% ethanol diluted 25 times was used as control groups at the same time. Three repetition was used in each treatment. The number of nymph produced and the animals dead was counted every two days.

Result & Discussion

Phytoecdysteroids content of methanolic extracts of *Ajuga* species

The Phytoecdysteroids in whole plant in *Ajuga multiflora* and in the aerial part of *A. linearifolia* identified by the HPLC analysis was listed in Table 2. This part was conducted by Dr. Josep Coll Toledano and Dr. Bela Darvas in Csic Department of Organic and Biological Chemistry, Barcelona, Spain.

Table 2. Phytoecdysteroids in *A. multiflora* and *A. Linearifolia*, mg · kg^{-1}

Phytoecdysteroids species	*Ajuga multiflora* whole plant	*A. linearifolia* aerial part
20-OH ecdysone	287	414
cyasterone	120	47
ajugalactone		33
3-acetate-20-OH ecdysone	14	-
2-acetate-20-OH ecdysone	21	-
total	442	494

Those data in table 2 shown that *A. linearifolia* containing more 20-OH ecdysone and Ajugalactone, *A. multiflora* containing more cyasterone, 3-acetate-20-OH ecdysone and 2-acetate-20-OH ecdysone. The total amount of phytoecdysteroids contained in *A. linearifolia* is 494 mg·kg^{-1} which was higher than that contained in *A. multiflora*.

Effects of extracts on larvae of *Clostera anastomosis* and *Stilpnotia candida*

In this experiment, the extracts drawn from A. multiflora and A. linearifolia were used to treat the larvae of *Clostera anastomosis* and *Stilpnotia candida*. Totally, 7 records have gotten in 21 days. Those records was summarized as Table 3 and 4.

Table 3. The mortality rate of *Clostera anastomosis* larvae treated by some *Ajuga* extracts

kind of extracts	times of dilution	number of larvae	number of dead larvae	rate of mortality (%)	modified mortality rate(%)
A. multiflora	25	60	52	86.67	85.03
	50	60	50	83.33	81.28
	100	62	38	61.29	56.53
A. Linearifolia	25	61	50	81.96	79.74
	50	59	49	83.05	80.97
	100	60	36	60.00	55.09
hexafluron	5mg·kg^{-1}	62	59	95.16	94.56
	10 mg·kg^{-1}	60	60	100.00	100.00
	20 mg·kg^{-1}	56	56	100.00	100.00
control		64	7	10.94	

Table 4. The mortality rate of *Stilpnotia candida* larvae treated by some *Ajuga* extracts

kind of extracts	times of dilution	number of larvae	number of dead larvae	rate of mortality (%)	modified mortality rate(%)
A. Multiflora	25	60	47	78.33	76.39
	50	60	48	80.00	78.21
	100	61	30	49.18	44.64
A. Linearifolia	25	58	56	96.55	96.24
	50	59	51	86.44	85.23
	100	61	37	66.66	57.15
hexafluron	5mg·Kg^{-1}	59	59	100.00	100.00
	10 mg·Kg^{-1}	59	59	100.00	100.00
	20 mg·kg^{-1}	60	60	100.00	100.00
control		61	5	8.20	-

From the results in Table 3 and 4 , we found out that those phytoecdysteroids contained in *A. multiflora* and *A. linearifolia* could lead the second instar larva of *Clostera anastomosis* and *Stilpnotia candida* to death. The modified mortality rate was from 44.64 % to 96.24%. Those extracts could kill more than 75% to 96.24% of the second instar larva of *Clostera anastomosis* and *Stilpnotia candida*, if they was only diluted

25 or 50 times. It seems that ,the killing ability to these two animals of those extracts was lower than that of 5 $mg \cdot kg^{-1}$ to 20 $mg \cdot kg^{-1}$ hexafluron. Five $mg \cdot kg^{-1}$ to 20 $mg \cdot kg^{-1}$ hexafluron could kill 94.56% to 100% second instar larvae of *Clostera anastomosis* and *Stilpnotia candida*.

Effects of extracts on larvae of *Tuberolachnus salignus*

Seven days was used to found out the effects of extracts of *A. multiflora* and *A. linearifolia* on larvae of *Tuberolachnus salignus*. The results of those observation were summarized as table 5.

Table 5. Effects of *Ajuga* extracts on larvae of *Tuberolachnus salignus*

Kind of extracts	Times of dilution	Number of female adults	Number of nymph produced	Number of dead nymph	Mortality rate (%)
A. multiflora	25	30	17	10	58.82
	50	30	12	6	50.00
	100	30	56	24	42.86
A. Linearifiora	25	30	14	6	42.86
	50	30	51	17	33.33
	100	30	39	15	38.46
hexafluron	5mg.Kg^{-1}	30	26	7	26.92
	10 mg.Kg^{-1}	30	14	5	35.75
	20 mg.Kg	30	7	3	42.86
control		30	74	13	17.57

The data in Table 5 indicated that the extracts made from *A. linearifolia* and *A. multiflora* could significantly reduce the amount of nympha produced by the female *Tuberolachnus salignus*. The number of the nympha produced after treating with those extracts was only 20% to 70% of that produced by the untreated group. The death rate of the newly produced nympha which treated with those extracts was among 33.33% to 58.82%. It was much higher than the death rate of the control group(17.57%). From the number of newly produced nympha and the death rate, we might found out that those extracts have similar effect with 5 $mg \cdot kg^{-1}$ to 20 $mg \cdot kg^{-1}$ hexafluron. The death rate listed in Table 5 was not very high. The reason for this phenomenon need further study.

References

1. Abubakirov, N. K. 1982. Ecdysteroids of flowering plants (Angiospermae). Proc. Indian. Science. Acad. 48 A (1): 122-138

2. Bergamasco, R. & Horn, D> H. S. 1983. distribution and role of insect hormones in plants. In:Downer,R G.H.& Laufer,H. eds. Endocrinology of insects. PP.627-654. Alan.R..Liss. Inc. New York.

3. Camps, F., Coll, J. Cortel, A. 1982. 29-norsengosterone and 29-norcyasterone. New C-28 Phytoecdysteroids from *Ajuga reptans* (Labiatae). Chem. Lett. 1313-1316

4. Camps, F., Coll, J. Molins,E. et al. 1985 a . 2-Acetyl and 3-Acetyl 29-norcyasterone, new minor phytoecdysteroids from *Ajuga reptans* (Labiatae). J. Chem. Res. M. 1:241-266

5. Camps,F., Coll, J. Dargallo, O.1985b Phytoecdysteroids from *Ajuga chmaepitys*. An.Quim., 81:74-75

6. Choum W.S. Lu. H.S. 1980 Growth regulators and silk production of *Bombyx mori* L. from phytogenous ecdysteroids. In: Hoffmann, J.A. ed. Progress in Ecdysone Research. pp. 281-297. Elsevier. Amsterdam.

7. Dargallo, O. 1985. Ph.D> Thesis, Fac. Pharmacy, Univ. Barcelona.

8. Ikan, R. Ravid, U. 1971a. The isolation and identification of cyasterone from *Ajuga iva*. Planta Medica . 20: 33-34

9. Ikan, R. Ravid, U. 1971a. The isolation and identification of cyasterone from *Ajuga chia* (Labiatae). Phytochemistry. 10: 1659-1661

10. Khafagy, S.M., Nazmi Sabri, N., El-Sebakhy, N., et al. 1979. A C-28 ecdysone-like substances from *Ajuga iva*. Planta. Medica, 35:184-185

11. Koreeda, M. Nakanishi, K. Goto, M. 1972. Ajugalactone, an insect moulting inhibitor as tested in the *Chilo* dipping method. J. Amer. Chem. Soc., 92: 7512-7513

12. Kubo,I., Klocke, J.A. Asano, S. 1981. Insect ecdysis inhibitors from the east African medicinal plant *Ajuga remota* (Labiatae). Agric. Biol. Chem., 45: 1925-1927

13. .Kubo,I., Klocke, J.A. Ganjian, I., et al. 1983. Efficient isolation of phytoecdysones from *Ajuga* plants by high-performance liquid Chromatography and droplet counter-current chromatography. Journal of Chromatography. 257.157-161

14. Matsumoto, T., Tanaka, N. 1991. Production pf phytoecdysteroids by hairy roots cultures of *Ajuga reptans var. atropurpurea*. Agric. Biol. Chem. 55: 1019-1025

15. Matsuka, T., Imai, S. Sakai, M., et al. 1969. Studies on phytoeddysone. Ann. Rep. Takeda Res. Labs. 28:221-271

16. Nazmi Sabri, N., Asaad, A., Khafagy, S. M. 1981. Isolation of four ecdysones from *Ajuga iva* roots and a rapid semiquantitative method for ecdysone determination. Planta Medica. 42:283-290

17. Usmanov, B.Z., Gorovits, M. B. Abubakhirov, N. K. 1975. Phytoecdysones of *Ajuga turkestanica*. III. Structure of turkesterone. Khim. Prir. Soedin. 466-470

18. Usmanov, B.Z., Rakhes,Y.V. Abubakhirov, N. K. 1978., Phytoecdysones of *Ajuga turkestanica*. VI. 22-acetyl-cyasterone. Khim. prir. Soedin. 215-219

19. Wessner, M., Champion, B. Girault, J.P. 1992. Ecdysteroids from *Ajuga iva*. Phytochemistry, 31:3785-3788

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